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POLLEN STERILITY IN RELATION TO THE GEOGRAPHICAL DISTRIBUTION OF SOME ONAGRACEAE¹

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(WITH PLATES XIX-XXI AND ONE FIGURE)

Before the development of methods of research in biology, the question of the origin of species was not debatable, since it was taken for granted that all had arisen as a result of special creation. LINNAEUS, however, in 1757 was convinced that this doctrine needed modification. His departure from the accepted hypothesis was brought about by the appearance of an intermediate form of *Tragopogon* resulting from a cross of *T. pratensis* and *T. porrifolius*, to which he gave the significant species name *T. hybridus*. Too much emphasis cannot be laid upon this fact, since it paved the way for the later and more complete conception of the fundamental laws of evolution.

From that time the occurrence of hybrid forms was recognized now and then, until the reality of their existence became universally admitted. During the later investigations of these plants, it became evident that hybridism was very closely correlated with that of a more or less sterile quality of the generative organs, in proof of which there has arisen a prodigious amount of literature. These numerous citations may well be considered as having established the general biological principle of sexual impotency resulting from earlier parental crosses. Since this form of degeneration is observed more readily in the microspores than in the megaspores, it naturally follows that the presence of a large and varying amount of infertile pollen may be considered as indicative of previous interbreeding of plants where such conditions obtain. Although this relation of pollen abortion to hybridism has become quite widely accepted, there appears to be still some doubt as to the validity of the general principle. Consequently, it may be advisable to mention a limited number of references in its support.

¹ Contributions from the laboratories of Plant Morphology of Harvard University No. 81.

In 1832 DUTROCHET (10) noticed the abortion of pollen grains in hybrids of *Prunus*. This relation, without doubt, received even greater recognition under DARWIN'S influence in 1859. The appearance of his *Origin of species* (5), in which he mentions the sterility of the offspring from hybrid parents, did much toward establishing this fact. The impaired fertility of garden varieties of plants likewise is valuable proof of this principle. The long-continued improvement of horticultural species by crossing has led to much investigation in this connection, and as a result many cases are on record where forms thus produced have become nearly or quite completely sterile. As an example of this form of sterility, conditions found in a garden hybrid of *Fuchsia* may be mentioned. This genus is well known to cross readily, and the resultant impotency of the sexual elements was observed by SMITH (22) in 1876. In his article he states that the pollen in garden fuchsias "is always variable." In a hybrid from *Fuchsia splendens* × *procumbens* he noticed that only about one-third of the pollen cells were triangular in form, and the remaining two-thirds were of a "peculiar shape" which (in view of our later studies) may be interpreted as an indication of a tendency to abortion in the anther. During the same year ANDERSON (1) states that only a few seeds in this species were potent, and a condition similar to this was cited by MEEHAN (21) in relation to cultivated varieties of *Viola* in 1889.

Since this article deals more especially with pollen conditions in the genus *Epilobium*, reference is made to HAUSSKNECHT'S (17) monograph of the genus, in which he mentions several hybrid forms. In his discussion of these crosses, he speaks of pollen sterility in relation to hybridization as follows: "Das beste Merkmal gewähren dann in diesem Falle die Pollenkörner, welche ich bei primären Bastarden in der kleinen und geschlossen bleibenden Anthere stets völlig verschrumpft vorfand. . . ." Statements similar to this were made by GUIGNARD (16) two years later in regard to hybridization in *Cistus*, *Begonia*, *Mespilus*, and *Crataegus*, the pollen of which he describes thus: "Les grains de pollen fertiles représentent environs le quart du nombre total, le rest est formé de grains de dimensions moindres, presque vides de protoplasma, ou n'ayant qu'un noyau au moment de la maturité de l'anthère." In his later

discussion of a special case, he concludes as follows: "Ce resultat m'apparaît donc fournir un argument important en faveur de la nature hybride de la plante en question."

The advent of the mutation theory of DEVRIES added a new factor to this discussion, the results of which have appeared in numerous publications. A brief mention of a very few of these results in regard to the correlation of hybridization and sexual impotency will suffice. DEVRIES (7) speaks of reduced fertility in crosses of *Oenothera biennis* × *muricata* and *Cytisus laburnum* × *purpureus*; BEER (4) showed that all the pollen of *Oenothera longifolia* did not reach maturity, as there was not sufficient nutritive material to carry all the grains to completion; TISCHLER (23) described a *Bryonia* hybrid resulting from a cross of *Bryonia alba* and *B. dioica* thus: "Die Pollenkörner sind sehr häufig (bei GREGORY fast immer) degeneriert, und von sehr ungleicher Grösse"; and GATES (12, 13, 14) describes the cytological development in relation to pollen degeneracy in some hybrid species of *Oenothera*.

BATESON (3) has been very influential in establishing the principle of sterility as a result of hybridism. Numerous references to his own observations add much weight to the value of the probability that previous crossing is indicated by an important condition of the microspores. This proof is augmented likewise by many citations of the results obtained by other investigators of this phenomenon. Conclusions similar to these were obtained by JEFFREY (18, 19, 20) as a result of studies of a large number of the vascular cryptogams, conifers, and angiosperms. The results of his researches may be summarized briefly as follows. In the more constant and distinct species, the pollen grains are generally equal in size, full of protoplasm, and show no indications of an arrested development. On the other hand, those forms which show a large degree of fluctuation and contain known hybrids, generally present a varying amount of impotent pollen cells. These opposing conditions are strictly in accord with the general principle just mentioned. Consequently, JEFFREY was led to infer that those species which are characteristically constant and exhibit perfect pollen should be considered as pure. In contrast to this type of spore development, he regarded those forms which are habitually variable

and which present impotent microspores as hybrid segregates, or hybrid species.

In view of this great quantity of evidence in favor of the coexistence of hybridism and sterility, together with a habitual lack of contradictory proof, it seems safe to assume that these conditions illustrate a general biological correlation. Consequently, whenever there appears a large percentage of impotent pollen on the anther, the natural conclusion is that this sterile state has been brought about by previous ancestral crossing.

These facts naturally lead to the question of the stability of hybrids in nature, which the morphological evidence of pollen sterility reveals as such. This question can likewise be answered in the same manner as the first, namely, by reference to cited examples. BATESON (3) in his discussion of this subject mentions a number of instances where hybrids have remained constant, instead of following the usual Mendelian principle. In regard to this phenomena he states as follows: "Literature of hybridization and heredity abounds with examples of hybrids which are said to have bred true, or, as we should say, without segregation"; DE VRIES (7) in a similar discussion of *Oenothera biennis* × *muricata* mentions constant hybrids which resemble one parent more than the other; and DAVIS (6) in speaking of crosses of *Oenothera biennis* and *O. grandiflora* says that hybrids were produced which resembled *O. Lamarckiana* to such a degree as to justify the consideration as a working hypothesis that *O. Lamarckiana* arose as a hybrid, and (to quote more directly) "it will be apparent to the reader that if the evidence should finally indicate *Lamarckiana* to be of hybrid origin, as a number of writers have already suggested, many of the so-called mutants are likely to be interpreted as segregates splitting off according to Mendelian expectations." Thus it is apparent that there are numerous quite constant hybrids which may be considered as segregated species.

Having discussed the morphological indications of the relation of pollen sterility to that of hybridization, and the proof of the stability of hybrids thus formed, it is evident that these two so well established principles must have some bearing upon the origin of certain races of plants. At present there are two conceptions

in regard to the evolution of species. There is that of DARWIN (5) which states that new forms represent a gradual transition of the existing structure through a slow process of a natural selection of minor variations. In opposition to this doctrine, there is the DeVriesian hypothesis of mutation, which explains the origin of new forms by the sudden acquisition or loss of characters (known as germinal variations), which are inheritable and breed true to type. The strongest argument against this latter conception is presented by the fact that the so-called mutants, especially those of *Oenothera*, show all the morphological evidences of hybrid origin. Those who support this view of evolution, however, state that the sterility of the pollen is as much an evidence of mutation as hybridization, but they fail to show the distinct difference between so-called mutants and those forms which may represent segregates derived from hybrid ancestors.

The argument that there is no tangible evidence of a gradual evolution is always advanced as a refutation against the Darwinian doctrine. As BARTLETT (2) states, "the few who still hold that the selection of continuous variations would suffice to bring about specific differentiation can bring forward little or no evidence to support their view. The evidence all points to the utmost fixity of organisms, aside from mutations." This point of view is based upon an apparent lack of transitional forms which are, in fact, characteristically absent in so far as the exterior structure is concerned. This conception owes its origin to the paucity of fossil plants, and the highly altered state of the external features of the remains which have been preserved as carbonized or petrified material and impressions. On the other hand, the internal organization of the woody stem shows abundant evidence of a gradual transition, in striking contrast to a characteristic lack of proof of the sudden appearance and later utmost fixity of anatomical structures. Although the general principles of plant morphology, that is, retention, reversion, and recapitulation, have become firmly established, and although their value in solving evolutionary problems is widely recognized, it may be advisable to mention a few examples which will show their application to the doctrine of a slow and continuous development.

Studies in this direction have shown that there has been a gradual transition of the pitted tracheid from that form characteristically present in the lowest of the vascular cryptogams, to that type normally found in the higher plants. The vessel also shows evidences of a continuous and uninterrupted evolution from that form found in the Gnetales to the compositaceous type of conducting elements. In the case of the wood ray, all the evidence points to this type of development. In the lowest forms of the vascular plants the ray is represented by a slight transformation of the tracheary elements, which gradually give place to the uniseriate parenchymatous plate, characteristic of the conifers. In the Gnetales and the lower angiosperms, this modification of the fibrous material occurs as a band of intermingled ray parenchyma and lignified fibers (the aggregate ray). By a gradual transition of the included tracheids or libriform fibers to storage cells (the first stage of which is seen in the septate condition of the fibers) the broad compound ray is formed. The wood ray reaches its highest development in the more advanced angiosperms, where the aggregate form has become spread out into scattered plates known as diffuse rays. Similar evidences of a gradual transition are seen in the development and distribution of wood parenchyma. This proof of a slow and unbroken evolution of these several woody elements is based, not only upon a study of fossil and existent forms, but also upon a comparison of the relative relation of progressive and conservative regions of the plant.

The value of this evidence of a gradual transition of woody structures cannot be overestimated, since it represents facts supporting general principles and distinctive of all the vascular plants from the lowest to the highest orders. Consequently, any factors of so universal occurrence as the development of the woody stem, conditions as equally characteristic of ancient as of modern forms, cannot logically be considered as "little or no evidence" in favor of a dilatory and uninterrupted change to a different type of structure. Furthermore, when the amount of proof exhibited by a study of all the main groups of the higher plants is weighed against the quantitative examples of so-called mutations, its numerical predominance is strikingly in

favor of the Darwinian rather than the DeVriesian hypothesis of evolution.

In contrast to this slow transformation in plants, there are larger and more inconstant variations which are brought about as a result of hybridization. These differences, thus formed, may follow a strict Mendelian ratio, or become segregates which breed true to type. This manner of fluctuation is so well known, however, that any further discussion of it is manifestly unnecessary.

Turning again to the consideration of the probable origin of species by mutation, as advanced by those who are in favor of this view, it is at least significant that by far the greater majority of the so-called species thus produced have descended from ancestry whose genetical purity is questionable. The genus *Oenothera* has been mentioned so frequently in this connection that it naturally deserves special consideration. This genus has long been known to hybridize freely, and also to have produced constant generations from crosses as a result of a segregation of characters. To place so much weight upon such precarious evidence as a distinction between segregates and so-called mutants, therefore, appears from the morphological standpoint, at least, to be unjustifiable.

Since the question of mutation so intimately concerns the Onagraceae, it seems advisable to consider the probable occurrence of hybrids in a few genera other than *Oenothera*, as indicated by the morphological evidence of pollen sterility. In this connection the following species will be discussed in respect to the probable relation of pollen sterility to hybridism and geographical distribution, namely: *Epilobium angustifolium* L., *E. latifolium* L., and *E. Dodonaei* Vill. of the subgenus *Chamaenerion*; *Epilobium hirsutum* L., *E. luteum* Pursh, and *E. obcordatum* Gray of the section *Lysimachion*; *Clarkia pulchella* Pursh, *C. rhomboidea* Dougl., and *C. Xanthina* Gray; *Oenothera serrulata* Nutt.; *Gongylocarpus fruticulosus* Benth. and *G. rubicaulis* Cham. and Schl.; *Diplandra lepezioides* Hook. and Arn.; and *Zauschneria californica* Presl.

The material for investigation was selected from herbarium specimens, representing widely separated stations throughout the greater part of the ranges of these species. Mature buds were chosen and treated with alcohol and NaOH, which caused them to

swell to their original size. The pollen thus treated was examined microscopically, and the relative amount of abortive grains (those which were shrunken, small, or empty) was determined by a more or less careful count of several fields of view. The difference between the fertile and the sterile microspores is perfectly distinct. The fertile ones appear as fully developed uniform spores filled with protoplasm; while those which have become degenerate are small, shrunken, and empty. Those buds, however, which showed less than 5 per cent of infertile pollen were considered as representative of genetically pure descent. This figure was chosen by DORSEY (9) after a careful investigation of *Vitis*. In this genus species of unquestionable ancestry were shown by a careful count never to contain more than this ratio as a result of physiological conditions.

From this general discussion of the relation of pollen sterility and variation in respect to hybrid formation, attention may be turned profitably to a more detailed discussion of the *Chamaenerion* group of *Epilobium*. It is of interest to note that DEVRIES (8) has stated that the pollen in *Epilobium* is "wholly fertile," but the writer's observations have led to different conclusions in regard to microspore conditions in this genus. *Epilobium angustifolium* L., with few exceptions, presents good pollen grains in the anthers chosen from plants which were collected in the more southerly part of its range. This condition is significant, since this region represents that part of its zone where it is outside the influence of *E. latifolium* L. This uniformly pure condition of the male generative cells is, without doubt, due to the fact that in the more southern stations *E. angustifolium* is practically a monotypic species, owing to the absence of an ally with which it is able to cross. On the other hand, where the "great willow herb" grows within the range of *E. latifolium*, the pollen shows a tendency to abortion in a varying degree.

A still more comprehensive understanding of the apparent relation of pollen sterility to geographical distribution can be obtained by reference to figs. 1-8, text fig. 1, and list I. Fig. 1 shows the pollen of *E. angustifolium* L. as it appears in plants from Auburn, New Hampshire. It will be observed that all the grains

are of uniform size and full of protoplasm. Fig. 2, representing a much enlarged view of pollen from the same region, shows clearly that there are no defective grains, but that all are equally well developed. Fig. 3 illustrates similarly perfectly developed microspores from the anther of a "fireweed" collected near Pocono Plateau, Pennsylvania, and the uniform structure of the pollen

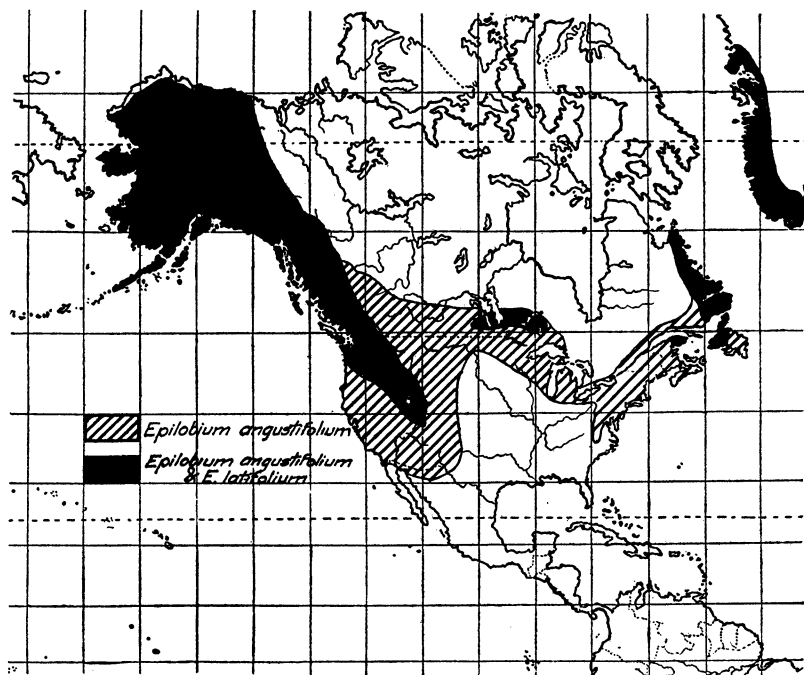


FIG. 1.—Relative distribution of *Epilobium angustifolium* L. and *E. latifolium* L.; solid black indicates region where the two species are coexistent; hatching indicates territory where *E. angustifolium* extends beyond range of *E. latifolium*.

from a West Virginia specimen is pictured in fig. 4. The two abortive grains among the perfectly formed ones in fig. 4, without doubt, have resulted from physiological conditions. In contrast to this perfect development of the pollen cells in these more southern representatives, fig. 5 represents the formation characteristic of the more northern regions where it grows within the range of *E. latifolium*. The pollen for this and the following illustration was chosen from a plant collected on Mount Albert in Gaspé

County, Quebec. Here, it will be observed, nearly one-third of the microspores are destitute of protoplasm, and all the grains, with a single exception, are much reduced in size. In fig. 6 similar indications of hybridism appear. In one case all the grains in a single tetrad, still remaining intact, have become defective. That like influences of degeneration may have caused many of the microspores of a Himalayan representative to stop growth in the early stages of development may be seen by reference to fig. 8. Several of the grains are perfectly developed, but a close observation will show also many small sterile pollen cells among those which have reached maturity.

A comparison of text fig. 1 and list I will show that there is a quite general tendency to abortion in that part of its range where *E. angustifolium* coincides with that of its nearest ally, *E. latifolium*. Defective microspores as an indication of hybridization are indicated by an asterisk in the list. In text fig. 1 those regions where these two plants are coexistent are represented in solid black. In contrast to this, those where *E. angustifolium* occurs alone are represented by hatching.

In view of the general principle of the correlation of pollen sterility and hybridism, previously mentioned, these facts are significant. From these studies it is apparent that when *E. angustifolium* grows within the range of its nearest ally, crosses take place which result in hybrid offspring. This relation is strikingly evident from the morphological standpoint at least, when one takes into account the presence of defective microspores in specimens chosen from that part of its habitat coterminous with that of *E. latifolium*. In contrast to this condition there is the more constant development of the pollen in buds selected from the more southern stations where *E. angustifolium* is practically monotypic.

LIST I

STATIONS OF *Epilobium angustifolium*, SHOWING RELATION OF PERFECT AND DEFECTIVE POLLEN TO LOCALITIES; DEFECTIVE POLLEN INDICATED BY AN ASTERISK

*Blanc Sablon River; Grand Falls, Newfoundland; East Avalon Peninsula, Newfoundland; *North of St. Paul's Bay, Cow Head, W. Newfoundland; *Curling, Bay of Islands, Newfoundland; *St. George's Pond, near Bay of

Islands, Newfoundland; Grindstone Island, Magdalen Islands, Quebec; *Seven Islands, Saguenay County, Quebec; *Lake Edward, Quebec; *Rivière du Loup, Timiscouata County, Quebec; *Mt. Albert, Gaspé County, Quebec; Table-topped Mountain, Gaspé County, Quebec; Little Métis, Quebec; Prince Edward Island; Kings Port, N.S.; Dead River, Somerset County, Me.; Fort Fairfield, Aroostook County, Me.; Jaffrey, N.H.; Mt. Washington, N.H.; Auburn, N.H. (figs. 1, 2); Mechanicsville, Vt.; Willoughby Notch, Vt.; Ashburnham, Mass.; Newton, Mass.; Southington, Conn.; Pocono Plateau, Pa. (fig. 3); Nescopeck, Luzerne County, Pa.; Blister Swamp, Randolph County, W.Va. (fig. 4); *Mungo Park, Nipigon Lake, Ont.; *Brown County, Wis.; Keweenaw County, Mich.; Banff Clearing, Alberta, Can.; Center City, Minn.; New Mexico; *Bridge Park, Carbon County, Wyo.; *Artist's Glen, Colo.; *Weber River, Wahsatch Mountains, Utah; *Revelstocke, Selkirk, Can.; Carbonate Landing, B.C. (50° 30' n. lat.); *Crane Lake, Assiniboia, Can.; *Emerald Lake, B.C.; Renfrew, Vancouver Island, Can.; *Silver City, Owyhee County, Idaho; Boise, Idaho; *Canyon County, Idaho; Clear Creek Canyon, Ormsby County, Nev.; Gunnerson Watershed, W. Cent. Colo.; Saturna Island, Humboldt Bay, Ore. Boundary; Clark Springs, Spokane, Wash.; Egbert Spring, Douglas County, Wash.; Swauk River, Kittitas County, Wash.; *Spalding, Ore.; Ten Sleep Lakes, Big Horn County, Wyo.; *Wahlamet, Douglas County, Ore.; Cold Water Canyon, San Bernardino County, Cal.; Waitesburg, Wash.; *Mt. Rainier, Wash.; Cascade Mountains (49° n. lat.); *Dutch Harbor, Unalaska, Aleutian Islands; *Makushin Bay, Unalaska; *Nazan Bay, Atka, Aleutian Islands; *Lake Lindman, Yukon River; *Disko, Greenland; *East Greenland; *Nain, Labrador coast; *Roma, Labrador coast; *Okak, Labrador; Hopedale, Labrador; Grand Lake, Newfoundland; *Newfoundland coast; *Table-topped Mountain, Quebec (fig. 7); *Mt. Albert, Gaspé County, Quebec (figs. 5, 6); *Turin, Marquette County, Mich.; Pagosa Springs, S. Colo.; San Francisco Mountains, Ariz.; Socorro County, N.M.; *Yellow Stone National Park; *Roger's Pass, Selkirk (51° 30' n. lat.); Nagai Island, Schumagin, Alaska; Tulare County, Cal.; *Bighorn Mountain, Wyo.; *Nashes Fork, Albany County, Wyo.; *Yukon, Can.; Anvik, Alaska; *Pluma, S.D.; Paradisino, Italy; *Lucetheshend, Surrey, England; *Sapporo, Japan; *La Buillard, Vosges Mountains, France; Louette St. Pierre, Belgium; Hupeh Province, Central China; *Rimkin, Tibet (fig. 8); Manchuria; Kurrum Valley, Afghanistan; *Island in Okhotsk Sea, N. Pacific; *Quichjock, Lapland; Hakone, Japan; *Reykjahled, N. Iceland; *Deuthfoss, Iceland; Harx, Germany.

Epilobium latifolium L. shows a similar condition of pollen sterility as a result of past crossing of its progenitors. Out of 32 plants examined, only 4 were found to contain structurally perfect cells, in contrast to 28 which showed abnormally developed micro-

spores, ranging from 10 to nearly 100 per cent in some cases. It is not surprising that this species should show a larger degree of abortion than the allied member of the subgenus *Chamaenerion*, since its entire range coincides with that of *E. angustifolium* as represented by the solid black portion of text fig. 1. List II shows the relative amount of sterile and perfect specimens of pollen. Fig. 9 illustrates pollen conditions in *E. latifolium* L. collected in Newfoundland. Reference to this figure will show that nearly all of the grains (those which are small, empty, and shriveled) are degenerate. Fig. 10 pictures the microspore formation in a plant from La Plata Canyon in Colorado. It will be observed that all the grains, with a single exception, are shrunken, and the fully formed one is abnormal in having more than the usual number of germination pores. Evidences of hybridism in an Alaskan *E. latifolium* are indicated in fig. 11. In this illustration the infertile grains appear as shrunken cells which are destitute of protoplasm. It is of importance to note in this connection that there is a general tendency in *E. latifolium* to abortion, and this condition is still more suggestive when it is observed that this plant, throughout its entire range, is coexistent with *E. angustifolium*.

LIST II

STATIONS OF *Epilobium latifolium*, SHOWING RELATION OF PERFECT AND DEFECTIVE POLLEN TO LOCALITIES; DEFECTIVE POLLEN INDICATED BY AN ASTERISK

*Disko, Greenland; *Godthaab, Greenland; *Tessiastuk, Greenland; *Hamilton Inlet, Indian Harbor, Greenland; *Roma, Labrador coast; *Valley of Exploits River, Newfoundland; Bay of Islands, Newfoundland; *Rope Cove, Newfoundland (fig. 9); *Grand Cascapedia River, Quebec; *St. John River, Gaspé County, Quebec; *Dartmouth River, Gaspé County, Quebec; *La Plata Canyon, Colo. (fig. 10); *Gunnerson Watershed, W. Cent. Colo.; *Graymount, Colo.; *Clear Creek, Colo.; *Buffalo River, Wyo.; *Mary Baker Lake, Mont.; *Fort St. Michael, Alaska; *Mt. Rainier, Wash.; *Seattle Wash.; *Shunagan Feld, Alaska; Lake Indian, Upper Yukon; *Chugackik Bay, Alaska; *Yakutat Bay, Alaska (fig. 11); *Selkirk, 118° 20' n. long. and 51° 45' n. lat.; *Juneau, Alaska; *Cold Foot, Yukon River, Alaska; *Cape Nome, Alaska; *Nazan Bay, Atka, Aleutian Islands; Akutan, Aleutian Islands; Glacier River, Unalaska, Aleutian Islands; *Mountains of Colorado.

It has already been pointed out that those species in which hybridism is habitual generally show a well marked variation. In this connection it may be well to mention the genera *Rubus*, *Rosa*, and *Crataegus*, whose fluctuation is very pronounced, in proof of which the lack of agreement in regard to their classification among systematic botanists is significant. This type of variability likewise is very noticeable in a study of any large amount of herbarium material of *Epilobium*, such as was observed by the writer in his examination of specimens at the Gray Herbarium. It is equally important in this connection to state that HAUSKNECHT (17) mentions 14 *formae* of *E. angustifolium*, 10 of *E. latifolium*, and 6 of *E. Dodonaei*. Thus it would appear that this subgenus presents capricious conditions of change similar to the genera just mentioned, which possess known hybrids and likewise present morphological evidences of pollen sterility as an indication of past interbreeding.

Epilobium Dodonaei Vill. is apparently subject to similar sterile conditions, since 2 out of the 3 specimens obtainable showed abortive pollen. This feature was also observed by Miss HOLDEN in Cambridge, England, as a result of an investigation of microspore conditions in relation to hybridism in this species and in *E. angustifolium* from Europe. The probability that crosses of this nature have taken place is strengthened by the fact that a hybrid from *E. Dodonaei* × *E. spicatum* is noted by HAUSKNECHT as described by HENNIGER.

Before turning to other species of the Onagraceae it seems advisable to summarize briefly the behavior of the species already discussed. It is apparent that the section *Chamaenerion* presents features which are characteristic of genera subject to hybridization in nature, as indicated both by sterility of the pollen and by a well marked variation. It is also evident that there is a distinct relation between this morphological proof of intercrossing and the geographical distribution of the species. In those regions where *E. angustifolium* has an opportunity to cross with its close allies, the anatomical proof appears conclusive that such crosses have taken place. Evidences of this phenomenon are still further strengthened by the fact that whenever this plant occurs sufficiently far south for crosses with other species of the subgenus *Chamaene-*

rion to be impossible, its microspores are usually perfectly developed and there appears little indication of abortion.

Hybrids have long been recognized in the subgenus *Lysimachion*, of which many are mentioned by HAUSSKNECHT, the names and parentage of which can be learned by reference to his monograph of the genus. Since this subgenus contains so many species, extending over a wide and continuous territory, a study of the pollen conditions in connection with their geographical distribution is impossible. It is significant, nevertheless, that the 3 species examined (*Epilobium hirsutum* L., *E. luteum* Pursh, and *E. obcordatum* Gray) showed evidences of pollen sterility. Similar microspore conditions were observed by JEFFREY (19) in a hybrid derivative of *E. hirsutum*.

Since the pollen in all of these species remains in the tetrad formation, it furnishes an excellent example of the relation of abortion to the number of grains derived from the mother cell. In a recent discussion of this subject DEVRIES suggested that, since three of the megaspores in the angiosperms are sterile, we might be led to infer that all the higher plants are hybrids, "at least on the material side." It is evident, however, that this condition has no bearing upon the question, since this type of degeneration represents a constant feature, and one which is in no way affected by intercrossing. In contrast to this form of spore abortion, that mode normally exhibited by *Epilobium hirsutum* and *E. obcordatum* may be mentioned. In anthers which show no evidences of abortion, the 4 microspores in the tetrad develop equally, and all are full and fertile at maturity. Conditions analogous to these were found to obtain in *Zauschneria californica* Presl., as indicated in figs. 17 and 18. Those plants, nevertheless, which show indications of being segregates from hybrid ancestors, possessed degenerate pollen grains, but these defective spores bore no constant relation to the original number in the tetrad. Fig. 12 shows the pollen formation in an apparent hybrid derivative of *Epilobium obcordatum* Gray. The spores at the left of the figure are quite fully developed in respect to the number in the tetrad. A single exception appears in the lower of the two groups, where a single grain has failed to reach maturity. The remaining clusters, it will be observed,

present more evidences of deterioration, and a further study of the illustration will show also that a large and variable number of the microspores have failed to attain normal development. Thus it is apparent that the process of degeneration in hybrid forms is in no way related to the processes of abortion of megaspores in the mother cell. The evidently pure species of the subgenus *Lysimachion* and the monotypic *Zauschneria* present uniformly perfect development of all the spores in the tetrad. In anthers showing morphological proof of earlier contamination, however, the number of abortive grains is in no way related to the number of microspores in the mother cell, since all the 5 possibilities of degeneration obtain.

The pollen conditions in *Clarkia* are not especially different from those already described for *Epilobium*. Of 16 specimens of *Clarkia pulchella* Pursh which were examined, 8 were found to contain nearly uniformly potent pollen cells. The remaining 8, however, showed distinct evidences of hybridism, as revealed by a large percentage of infertile pollen. *Clarkia rhomboidea* Dougl. showed the results of degenerating influences in a far less degree, and only 1 of 10 buds investigated presented indications of sterility. *Clarkia Xanthina* Gray exhibited features similar to those already mentioned in the discussion of spore conditions in *C. pulchella*. One-half of the buds studied presented defective microspores. Fig. 13 shows the type of pollen presented by plants possessing no morphological evidences of spore degeneration. In contrast to this uniform pattern, however, fig. 14 pictures the spore conditions in plants which have apparently been derived from hybrid ancestors. It will be observed that about half of the grains are empty and shriveled, and are strikingly unlike the fertile cells illustrated in the figure. As the character of pollen sterility in *Clarkia* differs in one of the essential features relative to *Epilobium*, it will not be discussed at length. Furthermore, since the several species occupy the same region, it is but natural to assume that they are more or less inclined to intercross freely. The offspring from these unions, accordingly, show a large and varying amount of defective microspores. There is, on the other hand, a perfect development of the pollen where such crosses have not taken place.

A consideration of pollen conditions in a few of the Onagraceae would be far from complete without a brief mention of some species of *Oenothera*. For this reason the distinct *Oenothera serrulata* Nutt. was chosen. This form is considered by some to be sufficiently well defined to be regarded as a separate genus, *Meriolix*. Of 22 specimens which were examined in the usual way, all were found to contain more than 50 per cent of abortive pollen grains. The defective grains, as can be seen by reference to fig. 15, are strikingly distinct when contrasted with the few fertile microspores. Likewise, the great variability in this genus, as well as an abundance of natural hybrids, are so well known and so universally accepted, that any further discussion of these phenomena in relation to sterility in hybrids would be superfluous. It is significant, nevertheless, that this species should show so large a percentage of defective pollen, and, as such, presents a condition strictly in accord with the general principle previously mentioned.

In contrast to the degenerate pollen development in *Oenothera*, conditions in the anthers of *Gonyglocarpus fruticulosus* Benth. and *G. rubricaulis* Cham. and Schl. should be mentioned. Both of these species show uniformly well developed microspores; there occur no indications of abortion in any of the grains. Similar spore formation was observed in *Diplandra lopezoides* Hook. and Arn., as illustrated in fig. 16. This shows all the cells to be equal in size and of uniform development. This naturally leads to the conclusion that the plants here mentioned have come from genetically pure ancestors. Although the genus *Gonyglocarpus* is considered by ENGLER and PRANTL (11) to be monotypic, 2 species were listed at the Gray Herbarium. The presence of two species, nevertheless, is unimportant from the standpoint of hybridism. *G. fruticulosus* is found in California, and *G. rubricaulis* grows near Vera Cruz, Mexico. The relative remoteness of these stations naturally precludes the impossibility of interfertilization, thus insuring in each a pure line of descent. The anatomical evidence of uncrossed species likewise supports this conclusion. All the pollen grains examined showed no indication of degeneration, but all were full of protoplasm and of equal magnitude.

Conditions of spore formation very similar to those described for the 2 species of *Gongylocarpus* were observed in *Zauschneria californica* Presl. It was possible to obtain only 4 specimens, but those presented microspore development such as might readily be expected in a monotypic genus. All the pollen cells were equal in size and fertility, as indicated by figs. 17 and 18. Fig. 17 represents a low magnification of the pollen of *Zauschneria*, and it will readily be observed that all the spores are perfectly developed. Fig. 18, a higher magnification, shows this fact even more conclusively. It is apparent also that all the spores in each tetrad reach maturity, and in no case do they show degeneration such as is habitual in 3 of the megaspores of the mother cell.

In respect to spore formation, this genus shows features parallel to those presented by the essentially monotypic *Gongylocarpus* and the geographically limited *Epilobium angustifolium*. The presence of entirely fertile pollen in these species is very significant. It is evident, of course, that hybridism is impossible. On this account all the specimens under consideration may be regarded as representative of pure lines of descent. That this purity of lineage is closely related to a perfect development of the pollen cells is strikingly evident from studies in this direction. The monotypic *Zauschneria* and the essentially monotypic species just cited generally present microspores which are perfect in every respect. Opposed to this type of spore formation, however, species possessing known or apparent hybrids generally contain pollen which is prone to abortion in varying degrees. Consequently, these 2 types of kindred phenomena show in a very conclusive manner the validity of the assumption that sterile pollen is reliable proof of hybrid lineage.

In the earlier paragraphs of this paper, the writer has attempted to show that there are certain general biological principles which have become firmly established. Naturally these principles have grown slowly, and have become far-reaching only in direct proportion to the amount of investigation carried on in relation to their application. Among the many laws thus formulated, that in regard to sexual impotency of hybrids is of the utmost importance in this discussion. The study of this problem, however, leads to

a consideration of the probable origin of species. As has already been stated, there are 2 distinct views in reference to this question. On the one hand, there is the idea of a gradual and continuous development; and on the other hand, the sudden and intermittent acquisition or loss of certain characters. These variations are said to breed true to type, and to form new and distinct species. The first of these conceptions receives very strong support from the characteristically gradual development of the woody elements in the lignified stem. These organs, as revealed by anatomical investigations, show a slow and continuous transition from the organization normally present in the lowest of the vascular cryptogams, to the form typical of the higher angiosperms. This support of the Darwinian hypothesis is still further strengthened by a characteristic lack of contradictory proof in the form of an intermittent course of development. Examples in favor of the opposing view represent isolated cases in certain groups of plants, of which the genus *Oenothera* has furnished the largest percentage. As has already been stated, there is a fundamental objection to the doctrine of mutation, especially in connection with *Oenothera*. This genus is well known to hybridize freely, and consequently the lineage of its so-called mutants must invariably be open to question.

This part of the discussion naturally leads into even narrower limits, that is, a recognition of hybrids in nature. In this connection there exists a general morphological proof of hybrid derivation. This condition is indicated by a distinct although variable percentage of abortive pollen grains in an anther. It should also be mentioned in this connection that most of the so-called mutants show this very evidence of hybrid descent.

It can readily be seen that the geographical distribution of a plant might have a direct influence on hybridization, and such has proved to be the case. *Epilobium*, especially the section *Chamaenerion*, furnishes an excellent example in this connection. *E. angustifolium* from those regions where it is essentially monotypic (the southern part of its range in the United States) presented generally perfect pollen. In contrast to this state, in specimens from the northern zone (where it is in contact with the allied

species *E. latifolium*) the pollen showed a strong tendency to abortion in varying degrees. Conditions similar to those were observed also in *E. latifolium*, and more generally in the subgenus *Lysimachion*, where there was quite habitual tendency to abortion of the microspores. This situation is due, without doubt, to the more constant presence of species between which crosses are possible.

In contrast to this condition, there is that presented by the 2 essentially monotypic species of *Gongylocarpus*, the geographically limited *Epilobium angustifolium*, and the strictly monotypic *Zauschneria californica*. In these plants there appears no evidence of pollen degeneracy, which (in view of the general principle of sterility in hybrids) is precisely the condition one would expect. It is, nevertheless, just as significant that these species which are unable to cross should present uniformly well developed pollen grains. This generally fertile state is in striking contrast to a habitual degenerate condition where there is frequent interfertilization. Thus it is apparent that reduced fecundity is valuable evidence of hybrid derivation, while a uniform development of the microspores is, other things being equal, as equally indicative of uncontaminated parentage.

From these studies of the Onagraceae in relation to geographical distribution, it is evident that impotent microspores are generally present in plants growing sufficiently near closely allied species to be the result of intercrossing. Opposed to this type of spore formation, there is a habitually perfect condition of the pollen chosen from plants growing in more isolated localities, and from monotypic species where interbreeding is manifestly impossible. Thus it is evident, from the morphological standpoint, that the Onagraceae intercross quite freely, and consequently there must be present many natural hybrids which greatly complicate genetical studies of species in this family.

Conclusions

1. It is apparent that abortive pollen, in a large though varying degree, is an indication of hybrid derivation.
2. There are many recognized hybrid segregates which have remained constant and true to type.

3. The relation of the geographical distribution in the subgenus *Chamaenerion* of *Epilobium* to hybridism is very pronounced. Whenever there is no possibility of intercrossing, the pollen is usually perfect. In contrast to this, there is a more general tendency to abortion in those regions where interbreeding is possible.

4. *Epilobium hirsutum*, *E. luteum*, *E. obcordatum*, and *Zauschneria californica* show conclusively that there is no relation between the number of microspores which may abort in apparent hybrid derivatives and the original number in the tetrad.

5. The essentially monotypic *Gongylocarpus*, the geographically limited *Epilobium angustifolium*, and the strictly monotypic *Zauschneria* present perfect pollen, a condition strikingly in accord with the general principle of hybrid sterility.

6. It is evident, on morphological grounds at least, that natural species crossing is a general feature among the Onagraceae, where such is possible in respect to geographical distribution and other factors.

In conclusion, the writer wishes to express his sincere thanks to Professors B. L. ROBINSON and M. L. FERNALD for permission to obtain specimens from the Gray Herbarium; and to Miss DAY, Librarian at the Gray Herbarium, for aid in securing literature. These investigations were carried on in the Laboratories of Plant Morphology of Harvard University, under the direction of Professor E. C. JEFFREY, and to him I am greatly indebted for advice.

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EXPLANATION OF PLATES XIX-XXI

FIG. 1.—*Epilobium angustifolium* L., showing entirely fertile pollen; ×75.

FIG. 2.—*E. angustifolium* L., showing perfectly developed pollen grains; ×125.

FIG. 3.—*E. angustifolium* L., showing no indication of abortion due to hybridism; ×150.

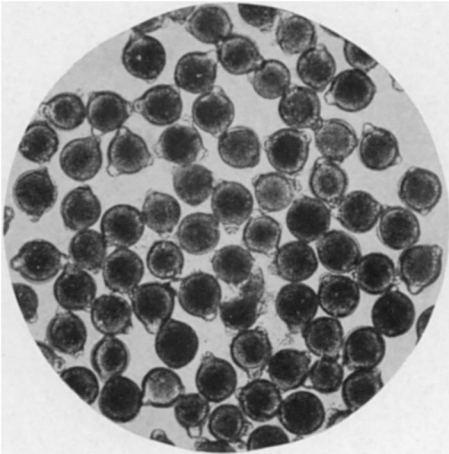
FIG. 4.—*E. angustifolium* L., showing fertile pollen; ×140.

FIG. 5.—*E. angustifolium* L., showing abortive pollen; ×125.

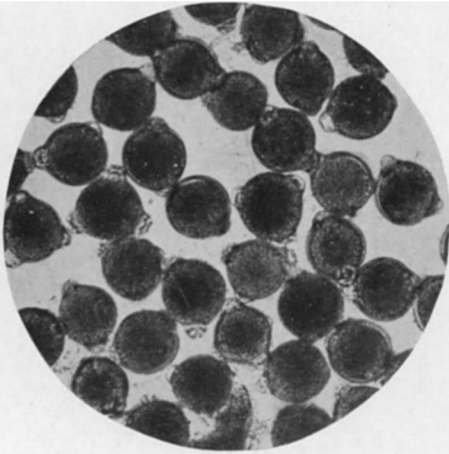
FIG. 6.—*E. angustifolium* L., showing fertile and abortive pollen, and a single tetrad in which all the grains are defective; ×125.

FIG. 7.—*E. angustifolium* L., showing indications of hybridism, as shown by the degenerate pollen grains; ×100.

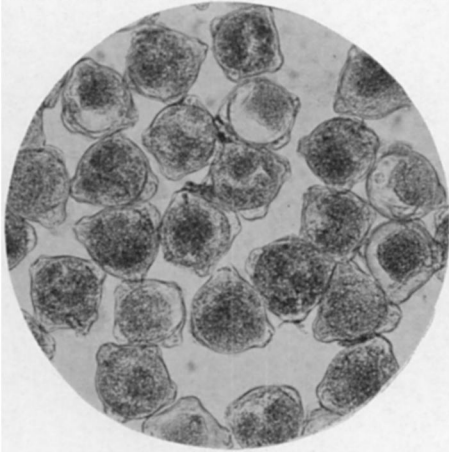
FIG. 8.—*E. angustifolium* L., showing fertile and infertile microspores; ×200.



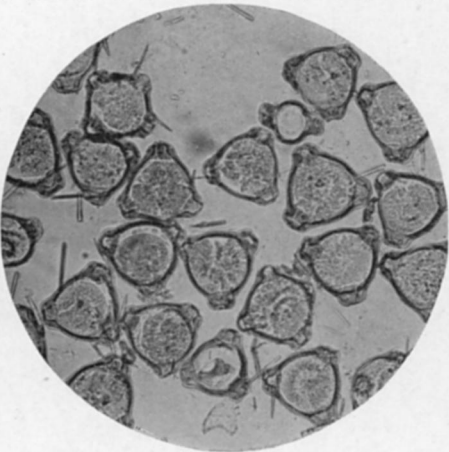
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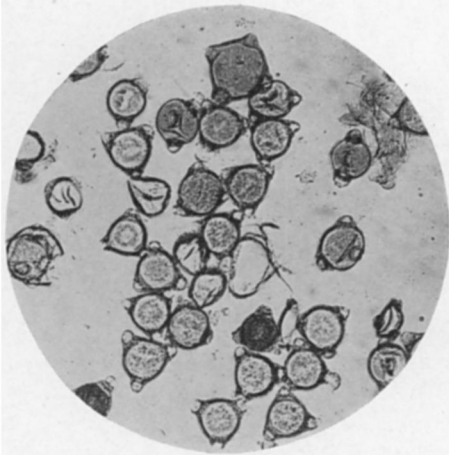
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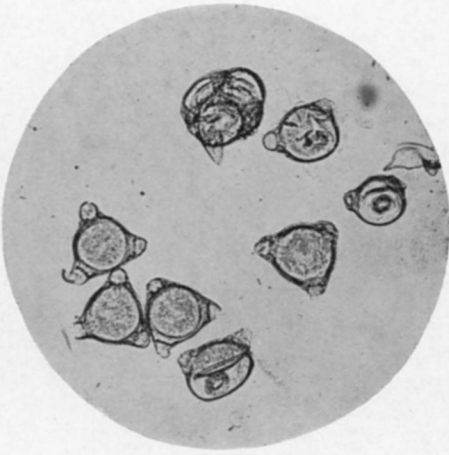
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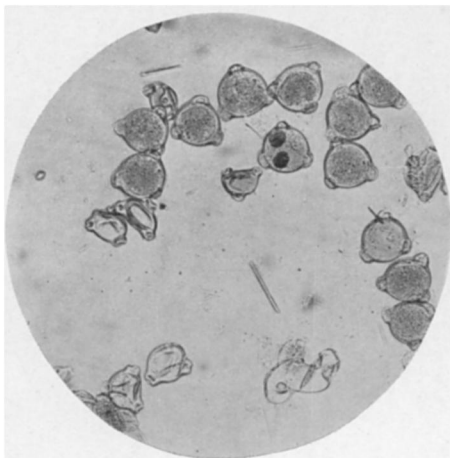
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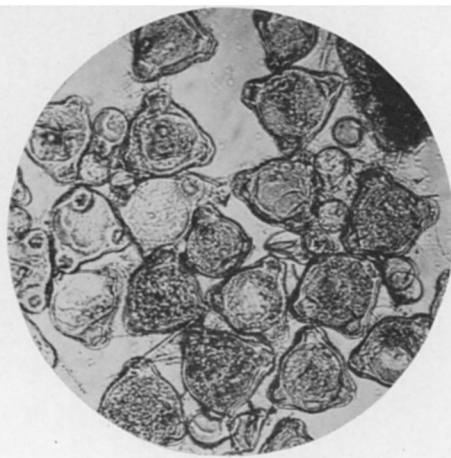
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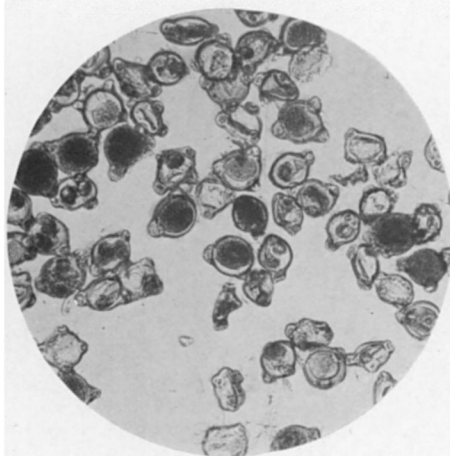
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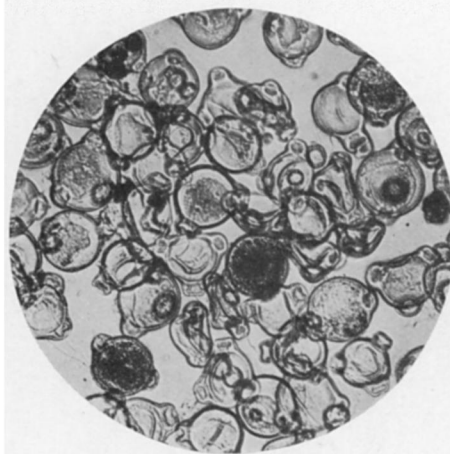
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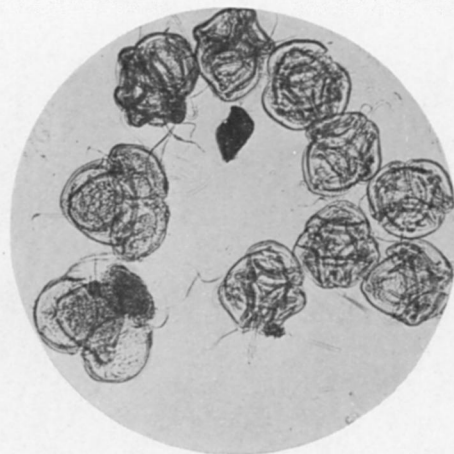
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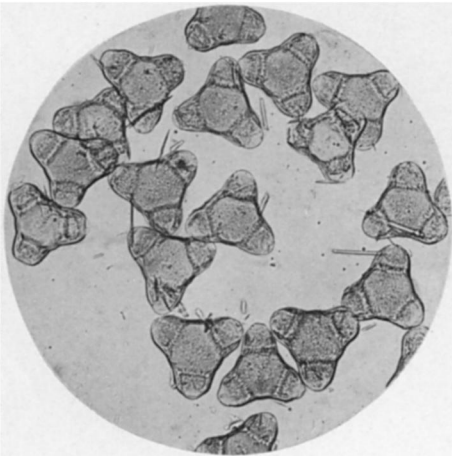
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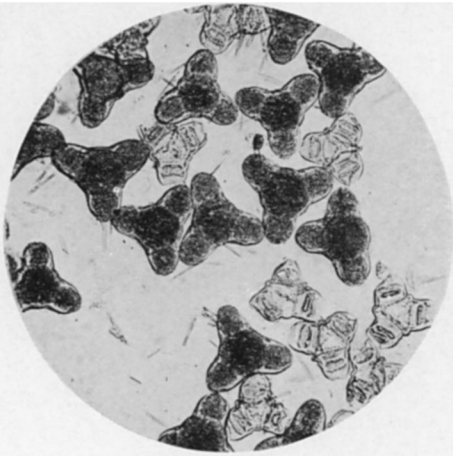
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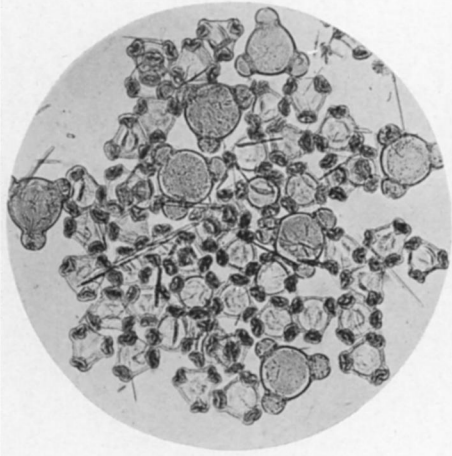
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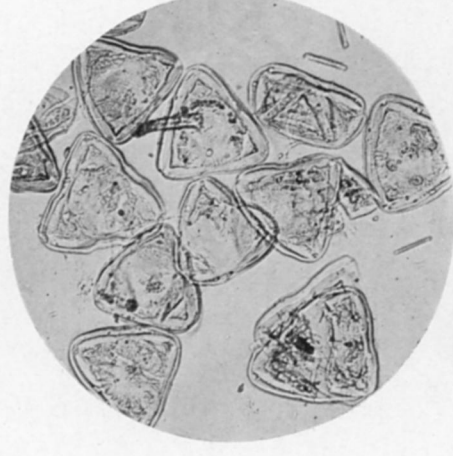
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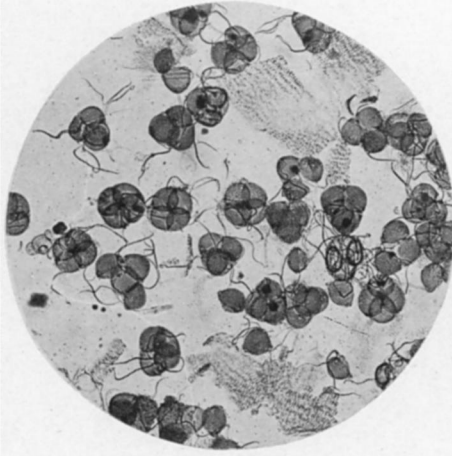
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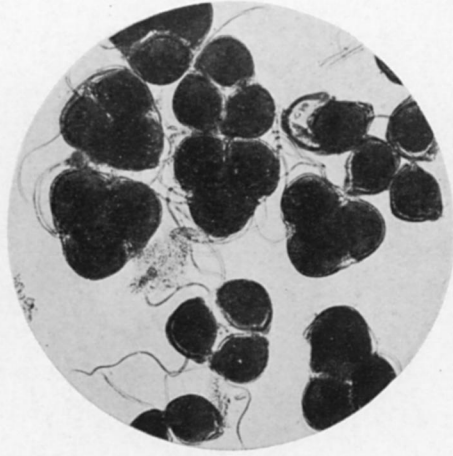
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FIG. 9.—*E. latifolium* L., showing evidences of abortion in a large percentage of the grains; $\times 75$.

FIG. 10.—*E. latifolium* L., showing defective pollen; $\times 150$.

FIG. 11.—*E. latifolium* L., showing perfect and imperfect pollen; $\times 125$.

FIG. 12.—*E. obcordatum* Gray, showing fertile and abortive pollen grains in relation to tetrad; $\times 125$.

FIG. 13.—*Clarkia Xanthina* Gray, showing uniformly developed pollen grains; $\times 125$.

FIG. 14.—*C. Xanthina* Gray, showing fertile and infertile pollen; $\times 125$.

FIG. 15.—*Oenothera serrulata* Nutt., showing relative size of fertile and degenerate pollen; $\times 125$.

FIG. 16.—*Diplandra lopezoides* Hook. and Arn., showing functional microspores; $\times 200$.

FIG. 17.—*Zauschneria californica* Presl., showing no indication of abortion in any of tetrads; $\times 50$.

FIG. 18.—*Z. californica* Presl., showing tetrads in which all grains are uniformly developed; $\times 125$.